

Contributions to a molecular phylogeny and systematics of *Anemone* and related genera (Ranunculaceae – Anemoninae)

Friedrich Ehrendorfer Rosabelle Samuel

(Department of Higher Plant Systematics and Evolution, Institute of Botany, University of Vienna, Rennweg 14, A-1030 Vienna, Austria)

Abstract Plastid *atpB/rbcL* intergenic spacer sequences were obtained from 21 selected taxa and one hybrid of Anemoninae (*Anemone*, *Pulsatilla*, *Hepatica*) and compared with *Ficaria* (Ranunculinae) as an outgroup. From the resulting matrix (1226 bp) a single most parsimonious tree was obtained (Fig. 1). The branching of this tree is confirmed by many informative indels and appears largely congruent with past plastid restriction analyses. Several new taxa are added. The monophyly of the Anemoninae and their early split into two major clades is supported: clade I with the chromosome base number $x = 8$, clade II with the reduced $x = 7$. Clade I is made up of the basal *Pulsatilla* and the *Rivularis* + *Vitifolia* groups. The *Multifida* group links to the crown groups *Coronaria*, *Blanda* and *Nemorosa*. Clade II consists of the basal *Dichotoma* group, followed by *Hepatica*, and finally by the N. Hemisphere *Narcissiflora* and the S. Hemisphere *Antucensis* groups as sisters. The problems of the Anemoninae ancestry, phylogenetic differentiation, and recent attempts for systematic classification are critically discussed. In view of the still incomplete sampling of DNA data, a conservative and informal approach to classification problems is recommended.

Key words Ranunculaceae; Anemoninae; *Anemone*, *Hepatica*, *Pulsatilla*; cpDNA; *atpB/rbcL* intergenic spacer sequences; Phylogeny; Systematics

In the most recent global systematic survey of the Ranunculaceae Tamura (1995) recognizes 7 genera within subfam. Ranunculoideae trib. Anemoninae of which *Anemone* (about 150 species), *Hepatica* (7 species), and *Pulsatilla* (about 40 species) are of relevance here. This treatment does not diverge in principle from the classical systematic survey of *Anemone* presented by Ulbrich (1905/06). In contrast, Starodubtsev (1991) used karyological, fruit anatomical and other data to support a splitting of *Anemone* into 7 additional genera. Already in 1994, Hoot *et al.* had published an important study on Anemoninae, mainly based on a plastid DNA restriction analysis and morphology, which was not considered by Tamura (1995). This DNA analysis supports Tamura's circumscription of Anemoninae as a monophyletic clade but demonstrates the origin of *Hepatica*, *Pulsatilla* and *Knowltonia* from within different *Anemone* groups. Thus, realizing the paraphyletic nature of the classical *Anemone* concept, Hoot *et al.* (1994) favor the lumping of all Anemoninae into a single genus, i.e. *Anemone* s. lat., and present a preliminary phylogenetic classification of this major clade.

In view of these conflicting interpretations, our objective is to present plastid *atpB/rbcL* sequences (Samuel *et al.*, 2001; Manen *et al.*, 1994; etc.) from several Anemoninae taxa obtained since 1996. This should help to improve our knowledge of the phylogeny and systematics of

the tribe within the framework of more extensive current research efforts, particularly by Sara B. Hoot and her collaborators at the University of Wisconsin (e.g., Schuettpehl *et al.*, 2001).

1 Materials and methods

1.1 Plant materials

Genera and species of Anemoninae and of *Ficaria* (basal Ranunculinae) as outgroup used for the present study are listed alphabetically in Table 1. Samples were either taken from plants in their natural habitats, from transplants into the Botanical Garden, University of Vienna (= HBV) or from lineages cultivated there.

The affiliation of the taxa is indicated in Table 1, first by the informal clades I and II (Ehrendorfer, 1995) which correspond, respectively, to *Anemone* subgen. *Anemone* and subgen. *Anemonidium* (Hoot *et al.*, 1994). Subordinate informal group names, mostly used already by Hoot *et al.* (1994) and Ehrendorfer (1995), are added and explained in more detail in the chapters 2.2 and 3.2. References to places of origin follow.

Voucher specimens of all provenances studied are deposited in the Herbarium of the Institute of Botany, University of Vienna (WU).

1.2 DNA extraction and amplification

Total DNA was extracted from fresh or silica-dried leaf material following the 2 × CTAB procedure of Doyle & Doyle (1987). The entire region of the non-coding intergenic spacer between *atpB* and *rbcL* was amplified using polymerase chain reaction (PCR). The highest PCR yield was obtained using the following conditions: 100 µL reaction contained 72.5 µL of sterile water, 10 µL of 10 × Taq polymerase reaction buffer, 2 mmol (4 µL of 50 mmol stock) magnesium chloride, 0.2 mmol (2 µL of 10 mmol stock) of each dNTP (total 8 µL), 0.25 mmol (2 µL of 50 mmol stock) of each primer (total 4 µL of forward and reverse), 2.5 units of Taq DNA polymerase, and 2 ~ 8 ng (1 µL of 2 ~ 8 ng/µL) of total DNA. Reaction mixtures were sealed with one or two drops of mineral oil to prevent evaporation during thermal cycling. Amplified fragments were checked on 1% agarose gel, and the amplified double stranded DNA fragments were purified using QIAquick (Qiagen, Ltd.) purification kit. We used the primers cited by Manen *et al.* (1994). The purified fragments were directly sequenced on an ABI 377 automated sequencer (PE Applied Biosystems, Inc.) using dye terminator chemistry following the manufacturer's protocols. Two cycle sequence reactions were performed for each template using each of the two PCR primers. The programs 'Sequence Navigator' and 'AutoAssembler' (PE Applied Biosystems, Inc.) were used to edit and assemble the complementary sequences. Each base position was examined for agreement between complementary strands.

1.3 Sequence alignment and phylogenetic analysis

Alignments were obtained using Clustal V (Higgins *et al.*, 1992), and these were then improved by eye. We used PAUP version 4.0b2a for heuristic phylogenetic analyses (Swofford, 1998). Many gaps were included in order to obtain proper alignment with the ingroup as well as the outgroup, but were not considered in the phylogenetic analyses. Tree searches were conducted under the Fitch criterion (unordered equal weights for all substitutions; Fitch, 1971) with 1000 random sequence additions, SPR (subtree pruning-regrafting) branch swapping, holding multiple trees per step (MULPARS on), but permitting only 10 trees to be held at each step to save time swapping on suboptimal trees. Fitch bootstrap percentages (Felsenstein, 1985) were calculated from 100

Table 1 Genera and species included in the present study in alphabetic order with references to clades, informal group names, and origins. HBV = Hortus Botanicus Vindobonensis, Botanical Garden of the University of Vienna. Further explanations in the text

Species	Clades and informal group names	Origin
<i>Anemone antucensis</i> Poeppig	II: Antucensis	Chile. Concepcion, Nahuelbuta, L. & F. Ehrendorfer, 24.01.98
<i>A. blanda</i> Schott et Kotschy	I: Blanda	Greece. Rhodos, F. Ehrendorfer, 20.04.1992
<i>A. canadensis</i> L.	II: Dichotoma	cult. HBV, F. Ehrendorfer, 15.06.1999
<i>A. caucasica</i> Willd. ex Rupr.	I: Blanda	Russia. Stavropol, S. Ziman, 04.1993
<i>A. coronaria</i> L.	I: Coronaria	France. Alpes Maritimes, S. Ziman N 1, 28.03.1997
<i>A. hortensis</i> L.	I: Coronaria	France. Alpes Maritimes, S. Ziman N 3, 28.03.1997
<i>A. hupehensis</i> Lemoine	I: Vitifolia	cult. HBV, F. Ehrendorfer, 15.06.1999
<i>A. multifida</i> Poir.	I: Multifida	USA. Colorado, S. Ziman CO6, 08.1997
<i>A. narcissiflora</i> L.	II: Narcissiflora	USA. Colorado, S. Ziman CO2, 08.1997
<i>A. nemorosa</i> L.	I: Nemorosa	Lower Austria. transplanted to HBV, F. Ehrendorfer, 12.04.1993
<i>A. nemorosa</i> L. × <i>A. ranunculoides</i> L. (= <i>A. × intermedia</i> Winkler)	I: Nemorosa	Lower Austria. transplanted to HBV, F. Ehrendorfer, 12.04.1993
<i>A. palmata</i> L. (2x)	I: Coronaria	France. Marseille, S. Ziman N4, 29.03.1997
<i>A. palmata</i> L. (4x)	I: Coronaria	Spain. Valencia, S. Ziman N15, 03.04.1997
<i>A. pavonina</i> Lam.	I: Coronaria	Greece. Olympia, F. Ehrendorfer, 02.04.1991
<i>A. ranunculoides</i> L.	I: Nemorosa	Lower Austria. transplanted to HBV, F. Ehrendorfer, 12.04.1993
<i>A. rivularis</i> Buch.-Ham. ex DC.	I: Rivularis	cult. HBV, F. Ehrendorfer, 15.06.1999
<i>A. sylvestris</i> L.	I: Multifida	Lower Austria. cult. HBV, F. Ehrendorfer, 15.06.1999
<i>A. tenuicaulis</i> (Cheeseman) Parkin & Sledge	II: Antucensis	New Zealand. South Island, Dunedin, U. Jensen, 14.01.1997
<i>A. virginiana</i> L.	I: Multifida	USA. Minnesota, S. Ziman MIN 1, 08.1997
<i>Ficaria verna</i> Hudson (= <i>Ranunculus ficaria</i> L.)	Ranunculinae (Outgroup)	Austria. Vienna, growing wild in HBV, F. Ehrendorfer, 20.05.2000
<i>Hepatica nobilis</i> Schreber	II: Hepatica	Lower Austria. transplanted to HBV, F. Ehrendorfer, 15.06.1999
<i>H. transilvanica</i> Fuss	II: Hepatica	Romania. cult. HBV, F. Ehrendorfer, 15.06.1999
<i>Pulsatilla grandis</i> Wenderoth	II: Pulsatilla	Lower Austria. cult. HBV, F. Ehrendorfer, 15.06.1999

replicates of simple sequence addition and TBR swapping.

The *atpB/rbcL* spacers from the plastid DNA of most taxa listed in Table 1 were completely sequenced. Only partial sequences were obtained for *Anemone caucasica* (and additional samples of *A. blanda* from Greece: Kerkyra and Patras/Mt. Klokos), and they have not been included in the

phylogenetic analysis and tree. All sequences obtained have been filed with GenBank, for the *atpB/rbcL* intergenic spacer under AF 386082→386100.

2 Results

2.1 Plastid DNA sequences

The length of the aligned *atpB/rbcL* spacer sequence is 1226 bp. Of the total number of characters 752 are constant and 234 are Parsimony informative. The most informative part of the matrix (bp 1 ~ 511 and 585 ~ 730) is reproduced in the appendix. The heuristic search generated a single most parsimonious tree with 794 steps, a constancy index of CI = 0.78 and a retention index of RI = 0.68. This tree is shown in Fig. 1, with number of substitutions above the branches (ACCTRAN optimization) and bootstrap percentages below. Removal of some ambiguous sequences from the matrix (e.g., bp 494 ~ 626, 644 ~ 784) did not change the topology of the tree.

As many as 41 informative indels (A-Z + a-o) were identified in the matrix; they are partially mentioned in the text with references to their bp positions (see Appendix). These indels considerably support our phylogenetic conclusions.

The complete marked matrix is not published here, but is available from the authors on request.

2.2 Sequence data and phylogenetic relationships

The results of our sequence analysis of the plastid *atpB/rbcL* intergenic region are shown as the one most parsimonious tree in Fig. 1. The basic congruence with the tree obtained from the plastid DNA restriction analysis (Hoot *et al.*, 1994; Fig. 1) is obvious. The monophyly of the Anemoninae, here compared with the outgroup *Ficaria* (a representative of the related Ranunculinae) is strongly suggested, just as with the outgroup *Clematis* (Clematidinae) used by Hoot *et al.* (1994).

Within Anemoninae our data also corroborate two major Anemoninae clades: The first (I), includes the taxa from the *Pulsatilla* to the *Nemorosa* group and is mainly characterized by its chromosome base number $x = 8$, the second (II) comprises the remaining taxa from the *Dichotoma* to the *Hepatica* group, all with $x = 7$. Because the basal branches of clade I (*Pulsatilla* group) and II (*Dichotoma* and *Hepatica* group) are strongly divergent and isolated, the bootstrap values alone are not convincing for these two major clades. Nevertheless, numerous characteristic indels support these and other branches of the two trees. Thus, indels F (bp 77 ~ 81), G (bp 82 ~ 87), W (bp 494 ~ 495), m (bp 827 ~ 833), and n (bp 836 ~ 839) characterize clade I, H + I (bp 83 ~ 87), K_{1-2} (bp 237 ~ 241), P (bp 300/307 ~ 319/321), and S (bp 407) clade II.

Within the major clade I, available sequence data place the representatives of groups *Pulsatilla* and *Rivularis* + *Vitifolia* into successive basal positions and demonstrate their considerable genetic distances from each other and the remaining clade I groups. Furthermore, *Pulsatilla* is separated from the rest of clade I by the indels N_1 (bp 261 ~ 274), Z (bp 644), c (bp 656), and e_2 (bp 679 ~ 694), the sister groups *Rivularis* and *Vitifolia* by the indel R (bp 330 ~ 335). Our data do not suggest other sister relationships in this basal assembly, as suggested by the plastid restriction analysis (Hoot *et al.*, 1994). Nevertheless, both data sets support the affinities between the Asiatic *Rivularis* and *Vitifolia* groups. These two are linked by the *Multifida* group to the crown groups *Coronaria* and *Blanda* + *Nemorosa*, which are (at least partly) held together by the indels B + C (bp 10/20 ~ 22/26, M (bp 270 ~ 274), and T_{3-5} (bp 452 ~ 456 / 457 / 462). In Hoot *et al.* (1994),

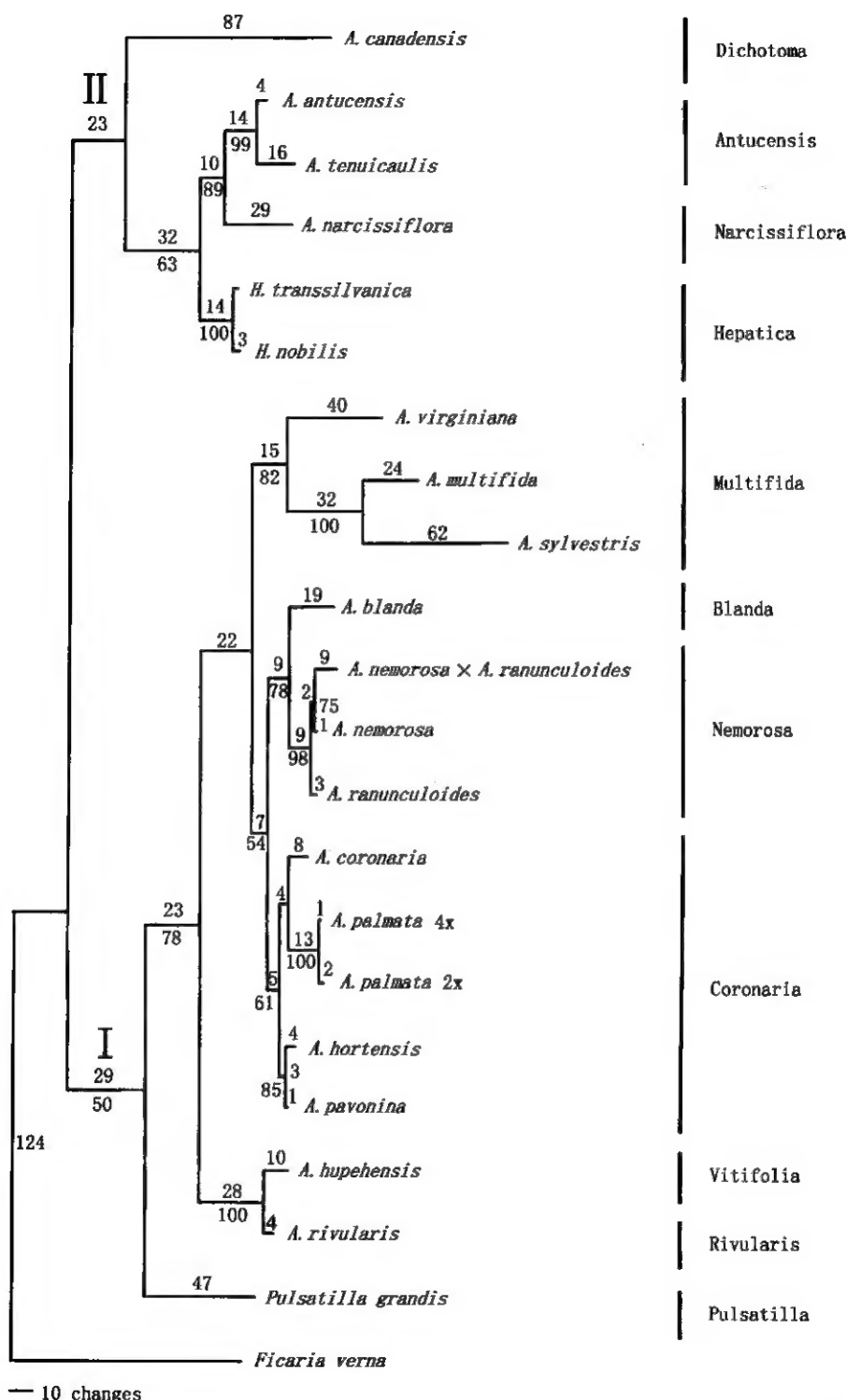


Fig. 1 Single most parsimonious tree for selected taxa of Anemoninae obtained from *atpB/rbcL* spacer sequences, using *Ficaria verna* (Ranunculinae) as outgroup. Number of substitution above, bootstrap percentages (> 50) below branches. Clade I and II, names of taxa and informal species groups are indicated.

the Blanda group is more closely attached to the Coronaria than to the Nemorosa group; the latter and the Multifida group appear as sisters.

Available *atpB/rbcL* sequences (and indel A, bp 3/5 ~ 26) confirm the circumscription of the N. American centered Multifida group with *A. virginiana*, and with *A. multifida* extending into S. America, *A. sylvestris* into Eurasia. The coherence of the Coronaria group is weakly supported (indel T₄₋₅: bp 452 ~ 457/462; M: bp 270 ~ 274 shared with the Blanda group). *A. hortensis* and *A. pavonina* are close and possibly only elements of one polymorphic Mediterranean species, whereas the often sympatric *A. coronaria* appears well separated. Even more isolated morphologically and in its plastid DNA [13 single steps and the indels C (bp 23 ~ 26), J (bp 209 ~ 214), T₅ (bp 458 ~ 462) etc.] is the rare and disjunct W. Mediterranean *A. palmata* whose affinities have not yet been studied by molecular methods. *A. palmata* is represented by 2x and 4x populations from S. France and SE. Spain; they differ little in plastid DNA.

The Blanda group, centered in the E. Mediterranean and the Caucasus area, consists of *A. blanda* (very close to *A. apennina*) and *A. caucasica*. Partial analyses of the *atpB/rbcL* spacer (not used for the tree Fig. 1) clearly show the separation of the two species by several point mutations and indels. There are only loose ties from the Blanda to the Nemorosa group (indel Q: bp 313 ~ 319 shared). The Nemorosa group exhibits a disjunct N. Hemisphere distribution, strongly linked to deciduous forest habitats. It is here represented by the predominantly European *A. nemorosa*, *A. ranunculoides*, and their sterile hybrid (= *A. × intermedia*). With respect to spacer sequences, this hybrid differs considerably from both parental species.

The major clade II of Anemoninae is also made up by two successive, basal, genetically rather isolated and only weakly supported branches, the Dichotoma and the Hepatica groups. According to our *atpB/rbcL* data the Dichotoma group is the first, confirmed by indels K₁ (bp 237 ~ 240), U₁₋₂ (bp 452 ~ 463), and o₅ (bp 927 ~ 931), and Hepatica the second branch, supported by the indels E (bp 13 ~ 22), O (bp 256 ~ 277), b (bp 651), d (bp 657/658 ~ 753), and l (bp 818 ~ 826), whereas this sequence is reversed in the restriction analysis (Hoot *et al.*, 1994). The Dichotoma group is constituted by the closely related *A. dichotoma* and *A. canadensis*. For the well separated Hepatica group we can add sequences for the two distinct Old World species *H. nobilis* and *H. transsilvanica*.

Sisters to Hepatica (connected by indel I: bp 83 ~ 87 and separated by indel f: bp 706 ~ 716) are the N. Hemisphere mountain Narcissiflora group and the S. Hemisphere group of Antucensis. Whereas the plastid DNA restriction data have already shown that the first belongs to the major clade II, this is new for the latter. Our matrix demonstrates that the Antucensis group is linked by a high bootstrap value to the Narcissiflora group and thus to clade II, but also considerably isolated by 14 substitutions and the indels D (bp 10 ~ 19) and N₂ (bp 261 ~ 287). Even if the circumscription of this Antucensis group against other C. and S. American species of *Anemone* (e.g., *A. helleborifolia*, *A. rigida*, *A. mexicana*, etc.) is still undecided (because DNA data are lacking for these taxa), the obvious affinities of the only New Zealand *Anemone*, i.e. *A. tenuicaulis* with *A. antucensis* from the Chilean Andes is now clearly established and confirmed by a high bootstrap percentage and common indels (see also Ehrendorfer & Samuel, 2000, and Schuettpelz *et al.*, 2001).

3 Discussion

3.1 Phylogenetic differentiation and evolutionary patterns

From the available DNA analytical data presented here and in the literature (Schuettpeitz *et al.*, 2001; Schuettpeitz & Hoot, 2000; Ehrendorfer & Samuel, 2000; Hoot *et al.*, 1995, 1994), the monophyletic origin of Ranunculaceae – Anemoninae and their subsequent split into two major clades is strongly supported. These clades have been called *Anemone* subgen. *Anemone* and subgen. *Anemonidium* by Hoot *et al.* (1994) or clade I and clade II by Ehrendorfer (1995). This split has been linked with a major reconstruction of the karyotype, a change in chromosome base number by descending dysploidy from $x = 8$ in clade I to $x = 7$ in clade II (Baumberger, 1970), and with a parallel increase (!) in nuclear DNA from I \rightarrow II (Rothfels *et al.*, 1966).

What is the possible character profile of the hypothetical ancestors of Anemoninae? The basis for such speculations are postulated character progressions from plesio- to apomorphic and the assumption that such ancestors might correspond to extant taxa situated close to the common base of clades I and II. These are members of the groups *Rivularis*, *Vitifolia* and *Canadensis*. Thus, Anemoninae ancestors may have been perennial herbs, growing in warm-temperate forests, with root-stock, tall and branched stems, compound basal, cauline and hardly differentiated opposite involucreal leaves, many-flowered inflorescences, flowers with sepals (but no petals), fruitlets \pm glabrous, with long beak and many-layered endocarp, pollen tricolpate, and chromosome base number $x = 8$. From such hypothetical ancestors one can imagine further differentiation into the clades I and II and their numerous species groups, often with world-wide radiations and adaptations into the most diverse habitats.

As Anemoninae have spread not only throughout the N. Hemisphere but at several occasions also have penetrated into the S. Hemisphere and dispersed between southern continents with taxa of clade I and II (see Schuettpeitz *et al.*, 2001), they probably date back to the late Cretaceous or the early Tertiary. The very different genetic distances of the branches in the phylogenetic Anemoninae trees (Fig. 1; Hoot *et al.*, 1994; Fig. 1) signal their differences in age and evolutionary phase, from the initial anagenetic (e.g., *A. sylvestris* in the *Multifida* group or the *Dichotoma* group) to the fully differentiating cladogenetic (e.g., the groups of *Coronaria* or *Nemorosa*), and finally to the depauperate and terminal stasigenetic phase (e.g., *A. tenuicaulis* in the *Antucensis* group).

3.2 Systematic problems and outlook

Considerations about a phylogenetic grouping and corresponding systematics of Anemoninae suffer from the still quite incomplete sampling with respect to DNA analytical data. Examples for these deficiencies (taken from Tamura, 1995) are several isolated, oligo- or monotypic taxa as the S. American genera *Oreithales* and *Barneoudia*, the monotypic SW. Chinese *Metanemone* as well as *Anemone* subgen. *Hepaticifolia*, subgen. *Rigida*, and subgen. *Rivularidium* p.p. from C. and S. America or subgen. *Anemoclema* and sect. *Begoniifolia* from E. and SE. Asia, all of which can not yet be placed with certainty into one of the two major clades (I and II) of Anemoninae. Apart from their different chromosome base numbers (I: $x = 8$, II: $x = 7$), reliable morphological or anatomical differential characters are still hardly available (see Hoot *et al.*, 1994) to separate these two clades, otherwise well supported by molecular data. This is probably due to parallel radiations into similar habitats by different clades, resulting in many homoplasies (Ehrendorfer, 1995).

Even with the limited DNA analytical data at hand it is worthwhile to compare the present mo-

molecular results with recent proposals for a taxonomic grouping of Anemoninae, particularly those by Starodubtsev (1991), Hoot *et al.* (1994), and Tamura (1995). With respect to the **generic level** and focussing on taxa for which DNA data are available, these proposals reach from the acceptance of the classical genera *Anemone*, *Hepatica*, *Pulsatilla*, and *Knowltonia* (Tamura, 1995) to their fusion into a single genus, *Anemone* s. lat. (Hoot *et al.*, 1994), or to the splitting of *Anemone* into 7 additional genera (Starodubtsev, 1991). The molecular evidence clearly proves that *Pulsatilla* and *Knowltonia* have originated from within the *Anemone* clade I in contrast to *Hepatica* which belongs to *Anemone* clade II. Thus, the classical concept of *Anemone* (Tamura, 1995) is unsatisfactory, because it is unbalanced, paraphyletic, and does not reflect the phylogenetic relationships within Anemoninae.

The generic concept of Starodubtsev (1991) is based on karyological, fruit anatomical and other observations and favors several smaller, clearly more homogeneous genera. Nevertheless, various conflicts with the molecular evidence remain. His narrow concept of *Anemone* L. s. str. includes, i. a., the groups of *Vitifolia*, *Multifida* and *Coronaria*, but excludes close relatives; the *Rivularis* group is placed into another genus, *Anemonidium* (see below), the *Blanda* and *Nemorosa* groups constitute the monophyletic genus *Anemonoides* Mill. [= *Anemone* subgen. *Anemonanthea* (DC.) Juz., p. p.: Tamura, 1995].

A new genus of Starodubtsev (1991) is *Pulsatilloides*; it corresponds only partly to Tamura's subgen. *Pulsatilloides* (DC.) Juz. and appears quite heterogeneous. Here he includes the S. African type species (*A. capensis*), the isolated Asiatic *A. begoniifolia* (with relatives) and *A. anemoclema*, both of uncertain position, and the *Obtusiloba* group (= sect. *Himalayica*). The molecular data, in conflict with this arrangement, demonstrate that *A. capensis* (together with *A. caffra*) form a monophyletic subclade of I, together with the "genera" *Knowltonia* in Africa, *Pulsatilla* in the N. Hemisphere, and the isolated *A. crassifolia* in Tasmania (Hoot *et al.*, 1994; Schuettpelz *et al.*, 2001), whereas sect. *Himalayica* belongs to clade II (see below).

Of particular complexity in the Anemoninae system of Starodubtsev (1991) is the genus *Anemonidium* (Spach) Holub. Its type is *Anemone dichotoma* which belongs, together with the close *A. canadensis*, to the *Dichotoma* group of clade II, related to the monotypic arctic *Richardsonii* group (both united as sect. *Anemonidium* in Hoot *et al.*, 1994; Schuettpelz *et al.*, 2001). Other elements of clade II and I included by Starodubtsev (1991) in his *Anemonidium* subgen. *Meridium* are the S. American *Antucensis* group (II), the heterogeneous *A. tenuicaulis* (II) + *A. crassifolia* (I) assembly, the E. Asiatic *Rivularis* group (I), and other C. and S. American taxa of uncertain position.

Another genus accepted by Starodubtsev (1991) is *Anemonastrum* Holub which corresponds to subgen. *Omalocarpus* (DC.) Juz. Here he fails to include the closely related sect. *Himalayica* (Ulbr.) Juz. (Schuettpelz *et al.*, 2001; Tamura, 1995; Hoot *et al.*, 1994), which is listed by him as part of the genus *Pulsatilloides* (see above). *Arsenjevia* and *Tamuria*, based on the E. Asiatic taxa *Anemone* sect. *Stolonifera* (Ulbr.) Juz. and sect. *Keiskea* Tamura, respectively, are two more new genera described by Starodubtsev (1991). Together, *Arsenjevia* and *Tamuria* correspond to what Hoot *et al.* (1994) have called *Anemone* subgen. *Anemonidium* sect. *Keiskea* and for which monophyly within clade II has been demonstrated (see also Schuettpelz *et al.*, 2001).

With respect to the **infrageneric systematics** of *Anemone* presented by Tamura (1995) a few remarks will suffice. His subgen. *Rivularidium* is correctly placed at the beginning with its rather

plesiomorphic character profile, but intermingles elements of clade I and II: ser. *Rivulares* (I, = *Rivularis* group) and ser. *Helleborifolia* (with *A. antucensis*; II), sect. *Crassifolia* (with *A. crassifolia*; I and *A. tenuicaulis*; II), and sect. *Richardsonia* (II). Subgen. *Omalocarpus* (II) correctly includes sect. *Omalocarpus* (= *Narcissiflora* group) and *Himalayica* (= *Obtusiloba* group), but sect. *Begoniifolia* (= *Begoniifolia* group) probably belongs to clade I (Hoot *et al.*, 1994). Subgen. *Anemonanthea* suffers from the homoplasies leading to the adaptive "Nemorosa syndrome" (Ehrendorfer, 1995): sect. *Hyalectryon* and *Tuberosa* (= *Nemorosa* and *Tuberosa* groups) are members of clade I, sect. *Keiskea* and *Stolonifera* (= *Keiskeana* and *Flaccida* groups) of II. Sect. *Eriocapitellata* (= *Vitifolia* group) and sect. *Erioccephalus* ser. *Rupicola* (= *Rupicola* group) as well as ser. *Virginianae* (= *Multifida* group, incl. of *A. sylvestris*) are better separated from the core of subgen. *Anemone*.

The new molecular data suggest only few changes in the concept of *Anemone* s. lat. proposed by Hoot *et al.* (1994). In subgen. *Anemone* (= clade I) the species *A. tenuicaulis*, *A. antucensis* (and other C. + S. American members of the *Knowltonia* group?) have to be transferred as a new group to subgen. *Anemonidium* (= clade II). The E. African *A. thomsonii* apparently has to be moved from sect. *Pulsatilloides* to the sect. *Anemone* (Schuettepelz & Hoot, 2000). The name "sect. *Anemospermus* DC." for the *Rivularis* and *Vitifolia* groups (clade I) is illegitimate, because it has been typified with *A. dichotoma* (clade II) and was subsequently also applied to several other groups.

In retrospect we can say that efforts to improve the phylogenetical arrangement of *Anemoninae* taxa have made considerable progress during the last decade. Nevertheless, there are still numerous gaps in the available molecular data. Until they have not been filled, it is probably better to refrain from using or creating new formal generic or infrageneric taxa and names, but rather stick to *Anemone* s. lat. or the classical concept, coupled with informal surveys of verified species groups.

Acknowledgements We are grateful to Dr. J.-F. Manen (Conservatoire et Jardin Botanique, Univ. Genève, Switzerland) for exploring and testing the possibilities for sequencing the *atpB/rbcL* spacer in *Anemone* and to Dr. Svetlana Ziman (Inst. Bot. Ukrainian Acad. Sc., Kiev) for plant material and cooperation.

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Appendix (continued)

[illegible]

Taxon

<i>A. virginiana</i>	TTCTTA-TTTCATTTCATCTTCATCATTTTC-TATTTA-----CACTTATTCTT--TCPTTGA-C---AAA
<i>A. multifida</i>	TTTITA-TTTCATTTCATCTTCATCATTTTC-TATTTA-----CACTTATTCTT--TTTTTGA-C---AAA
<i>A. sylvestris</i>	TTTTAA-TTTCATT-----CATCATTTTC-TATTT-----CCCTAATCTT--TTTTTG-C---CAA
<i>A. blanda</i>	TTCTTA-TTTCATTTCATCTTCATCATTTTC-TATTTA-----CACTTATTCTT--TCPTTGA-C---AAA
<i>A. ran X nem</i>	TTCTTA-TTTCAT-----CTTCATCATTTTC-TATTTA-----CACTTATTCTT--TCPTTGA-C---AAA
<i>A. nemorosa</i>	TTCTTA-TTTCAT-----CTTCATCATTTTC-TATTTA-----CACTTATTCTT--TCPTTGA-C---AAA
<i>A. ranunculoides</i>	TTCTTA-TTTCAT-----CTTCATCATTTTC-TATTTA-----CACTTATTCTT--TCPTTGA-C---AAA
<i>A. coronaria</i>	TTATTA-TTTCAT-----TTCATCATTTTC-TATTTA-----CACTTATTCTT--TCPTTGA-C---AAA
<i>A. hortensis</i>	TTATTA-TTTCAT-----TTCATCATTTTC-TATTTA-----CACTTATTCTT--TCPTTGA-C---AAA
<i>A. pavonina</i>	TTATTA-TTTCAT-----TTCATCATTTTC-TATTTA-----CACTTATTCTT--TCPTTGA-C---AAA
<i>A. palmata 2x</i>	TTATTA-TTTCAT-----CATTTTC-TATTTA-----CACTTATTCTT--TCPTTGA-C---AAA
<i>A. palmata 4x</i>	TTATTA-TTTCAT-----CATTTTC-TATTTA-----CACTTATTCTT--TCPTTGA-C---AAA
<i>A. hupehensis</i>	TTCTTA-TTTCAT-----CTTCATCATTTTC-TATTTA-----CACTTATTCTT--TCPTTGAAT---AAA
<i>A. rivularia</i>	TTCTTA-TTTCATTTTCATCTTCATCATTTTC-TATTTA-----CACTTATTCTT--TCPTTGAAT---AAA
<i>Pulsatilla</i>	TTCTCA-TTTCATTTCATCTTCATCATTTTC-TATTTA-----CACTTATTCTT--TCPTTGAAT---AAA
<i>A. canadensis</i>	TTTTAA-TTTCAC-TCATTTCCAT-----TAAATTT-----ACCTTATTTTC-----AAATTC---AAA
<i>A. antucensis</i>	TTTTTA-TTTCATCATTTCTATTTAAATTTA-----CACTTATTCTTTTTCTTTGAATAAA-AAG
<i>A. tenuicaulis</i>	TTTTTA-TTTCATCATTTCTATTTAAATTTA-----CACTTATTCTTTTTCTTTGAATAAA-AAG
<i>A. narcissiflora</i>	TTTTTAATT-CATCATTTCTATTTA-----CACTTATTCTTT-----GAATAAA-AGG
<i>H. transilvanica</i>	TTTTTA-TTTAATCATTTCTATTTAATTTAATTTAAATTTACACTTATCTTT-----GAATAAA-AAA
<i>H. nobilis</i>	TTTTTA-TTTAATCATTTCTATTTA-----CACTTATTCTTT-----GAATAAA-AAA
<i>Ficaria</i>	TGTTT--TCTTAT-----TTTATCATATC-TATTTACACTTCTTCTTT-----

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8888899999999000000000111111111222222222333333333444444444555555555
567890123456789012345678901234567890123456789012345678901234567

Taxon

<i>A. virginiana</i>	GAA--TTCTT?ATTTATA-----GCAC-TATTCACTATTCTATTTTCACATC-TAGGA--TTTA-CATATC-C
<i>A. multifida</i>	AAA--TTATAATTTATA-----GGGCTATTACCTATTCTATTTTCACATT-TAGGA--TTTTCCTTTTC-C
<i>A. sylvestris</i>	GAA--TTCTAATTTT?C-GGAAATTTTATTATGCGCCCTATT-CCCTATC--CAAT--TTTCCCATTTAGT
<i>A. blanda</i>	---TATTCATATTTATA-----GTACTATATTACTATTCTATTTTCACATC-TAGGA--TTTA-CATATC-C
<i>A. ran X nem</i>	---ATTCATATTTATA-----GTAC-TATTACCTATTCTATTTTCACATC-TAGGA--TTTACCATATA-C
<i>A. nemorosa</i>	---TATTCATATTTATA-----GTAC-TATTTACTATTCTATTTTCACATC-TAGGA--TTTA-CATATA-C
<i>A. ranunculoides</i>	--TATTCAATTT-ATA-----GTACCTATTACTATTCTATTTTCACATC-TAGGA--TTTA-CATATC-C
<i>A. coronaria</i>	--TATTCCTATTTATA-----GTAC-TATTTACTATTCTATTTTCACATC-TAGGA--TTT-ACATATA-C
<i>A. hortensis</i>	--TATTCATATTTATA-----GTAC-TATTTACTATTCTATTTTCACATC-TAGGA--TTT-CCATATA-C
<i>A. pavonina</i>	--TATTCATATTTATA-----GTAC-TATTTACTATTCTATTTTCACATC-T-GGA--TTT-CCATATAGC
<i>A. palmata 2x</i>	--TATTCATATTTATA-----GTAC-TATTTACTATTCTATTTTCACATC-TAGGA--TTTA-CATATA-C
<i>A. palmata 4x</i>	---TATTCATATTTATA-----GTAC-TATTTACTATTCTATTTTCACATC-TAGGA--TTTA-CATATA-C
<i>A. hupehensis</i>	-----TCI-----
<i>A. rivularias</i>	-CATATTCATATTACT-----ATG-----TTACTATTCTATTTTCACATC-TAGGA--TTTA-CATATA-C
<i>Pulsatilla</i>	-CATATTCATATTAC-----CTATTCACT-ATTCCATAATTTTCCCATCTAGGG-ATTTTACATATACA
<i>A. canadensis</i>	AAAA-TT---?------GGCAAATTCATATTACTATTCTATTTTCACATT-TAGGA--TTTA-CATATCCC
<i>A. antucensis</i>	-CATATTCATATTACTAT-----TCTATTACT-ACTATTCTATTTTCACATC-TAGGA--TTTACATATACA
<i>A. tenuicaulis</i>	-CATATTCATATTACTAT-----TCTATTACT-ACTATTCTATTTTCACATC-TAGGA--TTTACATATACA
<i>A. narcissiflora</i>	-CATATTC-----T-ACTATTCTATTTTCACATC-TAGGA--TTTACATATCCA
<i>H. transilvanica</i>	GCATATTCATATTACTAT-----TCTATTTTCACATC-TAGGA-TATTTAC-TTTACA
<i>H. nobilis</i>	-CATATTCATATTACTAT-----TCTATTTTCACATC-TAGGA-TATTTAC-TTTAC-
<i>Ficaria</i>	-C-----GGA-----TTTTCACATC--GAGG-ATTTA-CATATAC-

